


Protein purification, MST, and EMSAs

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The key micronutrient copper orchestrates broad-spectrum virus resistance in rice

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Detailed protocol

This protocol enables the detection of protein-ions binding affinity using MST.

1. The coding sequence of the DNA binding domain of OsSPL9 (SPL9 SBP) was inserted into the pGEX4T1 (GST tag, GE Healthcare, Chicago, IL, US vectors, and expressed as GST tag fusion proteins (GST-SPL9 SBP) in Transetta cells (DE3; TransGen Biotech, Beijing, China).
2. The fusion proteins were purified using glutathione sepharose 4B beads (GE Healthcare). The GST tag was removed by on-column Thrombin cleavage method, and the purified SPL9 SBP was dissolved in TBS buffer (20 mM Tris-HCl, pH 8.0, and 150 mM NaCl).
3. The MST assays was used for detecting interactions between SPL9 SBP and copper ions or calcium ions, the concentration of SPL9 SBP was held constant (1ug/ul), whereas the concentrations of copper or calcium ions were gradient diluted (ranging from 1000 uM to 0.03 uM with 2 fold gradient-dilution). The measurements were performed at 25 °C in buffer containing 20 mM Tris-HCl, pH 8.0, and 150 mM NaCl).
4. After a brief incubation for 5 min, the samples were loaded into MST standard glass capillaries. The measurements were performed at 25°C using 20% light-emitting diode power and 20% MST power in an MST machine (Monolith NT.LabelFree, NanoTemper Technologies, München, Germany).
5. Data analyses were performed using the NanoTemper Analysis and MO Affinity software provided by the manufacturer.
6. Note: When preparing the reaction buffer, it is important to consider that the Monolith NT.LabelFree measures MST via intrinsic tryptophan fluorescence (UV 280 nm). Most buffers, such as TBS or the buffer provided by MST, can be used. However, caution should be exercised to avoid contamination from nucleic acids or other substances, such as Triton X-100, which can cause absorption at 280 nm.

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1. Yao, S. and Li, Y. (2023). Protein purification, MST, and EMSAs. Bio-protocol Preprint. bio-protocol.org/prep2314.
2. Yao, S., Kang, J., Guo, G., Yang, Z., Huang, Y., Lan, Y., Zhou, T., Wang, L., Wei, C., Xu, Z. and Li, Y. (2022). The key micronutrient copper orchestrates broad-spectrum virus resistance in rice. Science Advances 8(26). DOI: [10.1126/sciadv.abm0660](https://doi.org/10.1126/sciadv.abm0660)

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